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# Long-term trends in CD4 cell counts, CD8 cell counts, and the CD4:CD8 ratio: ART Cohort Collaboration (ART-CC) Study

## Running head: Trends in CD4:CD8 ratio on ART

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## Abstract

**Objective:** Model trajectories of CD4 and CD8 cell counts after starting combination antiretroviral therapy (ART), and use the model to predict trends in these counts and the CD4:CD8 ratio.

**Design:** Cohort study of antiretroviral-naïve HIV-positive adults who started ART after 1997 (ART Cohort Collaboration) with >6 months of follow-up data.

**Methods:** We jointly estimated CD4 and CD8 count trends and their correlation using a bivariate random effects model, with linear splines describing their population trends, and predicted the CD4:CD8 ratio trend from this model. We assessed whether CD4 and CD8 count trends and the CD4:CD8 ratio trend varied according to CD4 count at start of ART (baseline), and, whether these trends differed in patients with and without virological failure more than 6 months after starting ART.

**Results:** A total of 39,979 patients were included (median follow-up was 53 months). Among patients with baseline CD4 count  $\geq 50$  cells/mm<sup>3</sup>, predicted mean CD8 counts continued to decrease between 3 and 15 years post-ART, partly driving increases in the predicted mean CD4:CD8 ratio. During 15 years of follow-up, normalisation of the predicted mean CD4:CD8 ratio (to >1) was only observed among patients with baseline CD4 count  $\geq 200$  cells/mm<sup>3</sup>. A higher baseline CD4 count predicted a shorter time to normalisation.

**Conclusions:** Declines in CD8 count and increases in CD4:CD8 ratio occurred up to 15 years after starting ART. The likelihood of normalisation of the CD4:CD8 ratio is strongly related to baseline CD4 count.

**Keywords:** combination antiretroviral therapy, CD4 cell count, CD8 cell count, CD4:CD8 ratio, HIV

## Introduction

Combination antiretroviral therapy (ART) has led to substantial increases in the life-expectancy of HIV-positive individuals [1]. Substantial declines in rates of AIDS have led to increased interest in non-AIDS, age-related diseases such as cardiovascular diseases, non-AIDS cancers, kidney disease, and neurocognitive decline [2-7], rates of which are higher than in the general population [8]. HIV infection leads to persistent immune activation and inflammation, which may accelerate immuno-senescence (deterioration of the immune system due to ageing) [9]. In the general population, a low CD4:CD8 ratio is a surrogate marker for immuno-senescence and an independent predictor of all-cause mortality [10,11]. Among HIV-positive individuals, low CD4:CD8 ratio has been associated with higher levels of immuno-senescence and inflammation, though the results regarding whether a low or inverted CD4:CD8 ratio predicts non-AIDS-related morbidity and mortality have been conflicting [12,13].

The benefits of ART for recovery of CD4 cell counts ('CD4 counts') are well documented [14-16]. However, few studies have investigated long-term trends in CD8 cell counts ('CD8 counts') [17-21] and in CD4:CD8 ratio [17-24] after starting ART, with almost all based on small (<150) or moderate (<2000) sample sizes. Given the potential health implications of elevated CD8 counts and CD4:CD8 ratios below 1 [19,20;25], more information is needed about their long-term trends in treated patients.

Our aims were to quantify long-term trends in CD8 counts and CD4:CD8 ratios, up to 15 years after starting ART, in a large cohort of antiretroviral-naïve individuals starting ART, and assess the impact of baseline CD4 count on these trends.

## Methods

### Study patients

The ART Cohort Collaboration (ART-CC) is an international collaboration between prospective cohort studies of HIV-positive individuals residing in Europe and North America, described elsewhere [26] and at [www.art-cohort-collaboration.org](http://www.art-cohort-collaboration.org). Cohorts enrolled HIV-positive ART-naïve individuals aged  $\geq 16$  years starting treatment with a combination of at least three antiretroviral drugs. All cohorts provided anonymized data on a predefined set of demographic, laboratory and clinical variables, which were then pooled and analysed centrally. The NHS Health Research Authority South West, Cornwall and Plymouth Research Ethics Committee, UK, approved the study (REC reference 12/SW/0253).

Eligible patients were antiretroviral-naïve, started ART after 1997, had at least one CD4 and CD8 measurement within the baseline period (defined as 90 days before to 6 days after starting ART), and one or more CD4 and CD8 measurements 6 months after starting ART.

### Statistical analyses

CD4 and CD8 counts were natural-log transformed (zero counts were set to 1), to stabilize the variance and meet normality assumptions. When modelling the relationships of log-CD4 and log-CD8 with time, we considered fractional polynomials of one to four degrees with powers -2, -1, -0.5, 0, 0.5, 1, 2, 3 (power zero is interpreted as natural-log transformation) and linear spline models with 2-5 knots. We compared model fit using the Bayesian Information Criterion (BIC) and the percentage of fitted values within 5% of observed values. We jointly modelled log-CD4 and log-CD8 using a bivariate random effects model. We included patient-level random effects for the intercept and trajectory terms, thus allowing trajectories

to vary between patients. We allowed patient- and measurement-level residuals to be correlated, thus allowing associations between CD4 and CD8 trajectories at both patient and measurement levels. Using the best fitting model, we estimated predicted means of log-CD4 and log-CD8, which when exponentiated became geometric means of CD4 and CD8 counts respectively. We calculated the difference between predicted means of log-CD4 and log-CD8, which were exponentiated to derive predicted geometric mean CD4:CD8 ratio. CD4:CD8 ratios  $>1$  were defined as normalised.

Patients were classified by their baseline CD4 ( $<25$ , 25–49, 50–99, 100–199, 200–349, 350–499,  $\geq 500$  cells/mm<sup>3</sup>), and CD8 ( $<500$ , 500–749, 750–999,  $\geq 1000$  cells/mm<sup>3</sup>) counts. Patients with multiple measurements within the baseline period were classified using the measurement closest to their ART start date.

We included covariates sex, age at start of ART, risk transmission group, ethnicity, and baseline CD4 and CD8 groups. To allow CD4 and CD8 trajectories to vary between baseline CD4 groups, we included interactions between baseline CD4 group and the intercept and trajectory terms.

Virological failure was defined as a HIV RNA measurement exceeding 1000 copies/mL, regardless of whether a patient had interrupted treatment. We generated a binary, time-independent variable denoting whether, from 6 months after starting ART, patients experienced at least one virological failure ('virologically unsuppressed') or all viral load measurements were  $\leq 1000$  copies/mL ('virologically suppressed'). We predicted geometric means of CD4 and CD8 counts separately among patients classified as virologically

suppressed and unsuppressed by including three-way interactions between the intercept and trajectory terms, baseline CD4 group and viral suppression indicator.

In sensitivity analyses, we predicted geometric mean CD4 and CD8 counts separately among patients who started treatment before 2004 and from 2004 onwards, and included the 6% of patients who had no CD4 or CD8 measurements 6 months post-ART.

Analyses were conducted using STATA/MP version 14 [27], with the runmlwin command [28] to run software MLwiN (version 3.01) [29] from within Stata.

## Results

Of 110,098 patients included in ART-CC up to 31<sup>st</sup> December 2013, 15% were excluded because they started ART before 1998 or before entering the study, 43% without CD4 or CD8 measurements within the specified baseline period, and 6% because they had no CD4 or CD8 measurement after 6 months since starting ART. Table 1 presents characteristics of the remaining 39,979 eligible patients according to baseline CD4 count. Most were men, approximately 40% were heterosexual, and the median age at start of ART was about 40 years. Higher baseline CD4 count predicted higher baseline CD8 count, with substantial reductions in median CD8 count as baseline CD4 count decreased from 100-199 cells/mm<sup>3</sup> to <25 cells/mm<sup>3</sup>.

Figure 1 shows trajectories of geometric mean CD4 count, CD8 count and CD4:CD8 ratio according to baseline CD4 group, predicted using the best fitting models (linear splines, with knots at 1, 21, 48 and 75 months for CD4 count, and at 6 weeks, 9, 36 and 66 months for CD8 count). Among patients with baseline CD4 count <200 cells/mm<sup>3</sup>, mean CD8 counts



increased steeply during the first 6 weeks of treatment, slowly decreased between 6 weeks  
 and 9 months post-ART, slowly increased between 9 months and 3 years, and slowly  
 decreased between 3 and 6 years. For example, among patients with baseline CD4 count  $<25$   
 cells/mm<sup>3</sup> the estimated ratios of geometric mean CD8 count per month were 1.6724 [95%  
 confidence interval 1.6509,1.6938] during the first 6 weeks of treatment, 0.9971  
 [0.9948,0.9994] between 6 weeks and 9 months post-ART, 1.0045 [1.0038,1.0051] between  
 9 months and 3 years, and 0.9969 [0.9963,0.9975] between 3 and 6 years. Lower baseline  
 CD4 count predicted larger declines in CD8 count between 3 and 6 years post-ART (see  
 Appendix-table 1). Among patients with baseline CD4 count 200–499 cells/mm<sup>3</sup>, mean CD8  
 counts slowly increased during the first 6 weeks, steeply decreased from 6 weeks to 9 months  
 post-ART and then slowly decreased from 9 months post-ART. For example, among patients  
 with baseline CD4 count 200–349 cells/mm<sup>3</sup>, the estimated ratios of geometric mean CD8  
 count per month were 1.0076 [1.0022,1.0130] during the first 6 weeks, 0.9805  
 [0.9795,0.9814] between 6 weeks and 9 months, 0.9988 [0.9985,0.9991] between 9 months  
 and 3 years, 0.9993 [0.9991,0.9996] between 3 and 6 years, and 0.9989 [0.9986,0.9991] from  
 6 years to end of follow-up. Among patients with baseline CD4 count  $\geq 500$  cells/mm<sup>3</sup>, mean  
 CD8 counts steeply decreased during the first 9 months post-ART, levelled-off between 9  
 months and 3 years, and then slowly decreased thereafter. From 6 years post-ART, mean  
 CD8 count plateaued among patients with baseline CD4 count  $<50$  cells/mm<sup>3</sup>, but continued  
 decreasing among patients with baseline CD4 count  $\geq 50$  cells/mm<sup>3</sup>.

Mean CD4 counts increased throughout follow-up, except for patients with baseline CD4  
 count  $\geq 350$  cells/mm<sup>3</sup> in whom mean CD4 counts plateaued or slowly decreased between 21  
 months and 6 years post-ART. Among all patients, the mean CD4:CD8 ratio trajectory  
 followed the same pattern as the mean CD4 count, indicating that changes in CD4:CD8 ratio

were mainly driven by changes in CD4 count. However, during periods of decreasing CD8 count, relative increases in the mean CD4:CD8 ratio were higher than those of mean CD4 count. Among patients with baseline CD4 count  $<50$  cells/mm<sup>3</sup>, increases in mean CD4:CD8 ratio 6 years post-ART were only driven by CD4 count increases, whilst among the remaining patients increases in the mean CD4:CD8 ratio were driven by both increasing CD4 counts and decreasing CD8 counts. During the 15-year follow-up period, the mean CD4:CD8 ratio only exceeded 1 among patients with baseline CD4 count  $\geq 200$  cells/mm<sup>3</sup>: higher baseline CD4 count predicted a shorter time to mean CD4:CD8 ratio  $>1$ .

Trends in mean CD8 count and CD4:CD8 ratio were similar between patients who were and were not virologically suppressed from 6 months post-ART (appendix-tables 2 and 3). However, changes in mean CD8 counts were more beneficial (smaller mean increases and larger mean decreases), and increases in the mean CD4:CD8 ratio were larger, among virologically suppressed patients compared to those not suppressed.

Trends in mean CD4 counts, CD8 counts and CD4:CD8 ratio were similar among patients who started treatment before 2004 and those who started 2004 onwards. However, patients starting treatment from 2004 onwards had higher mean CD4 counts, lower mean CD8 counts and higher CD4:CD8 ratios throughout follow-up (results not shown). Including the 6% of patients who did not have any CD4 or CD8 measurements 6 months post-ART had minimal effect on the results (results not shown).

## Discussion

Using data from a large collaborative study of HIV-infected individuals residing in Europe and North America, we found among patients with baseline CD4 count  $\geq 50$  cells/mm<sup>3</sup>,

predicted mean CD8 counts continued to decrease between 3 and 15 years post-ART, partly driving increases in the predicted mean CD4:CD8 ratio. Lower baseline CD4 count predicted higher increases in the mean CD4:CD8 ratio during the first 6 years since start of ART. Nonetheless, even 15 years since start of ART, the mean CD4:CD8 ratio did not normalise among patients with baseline CD4 count  $<200$  cells/mm<sup>3</sup>.

Few studies have reported long-term trends in post-ART CD8 counts. Two years post-ART, two studies reported similar findings to ours, of continued declines in CD8 counts [18;21], whilst three studies reported stabilised CD8 counts at elevated levels (approximately 600 to 900 cells/mm<sup>3</sup>) [12;17;19]. However, sample sizes in these studies were small compared to ours ( $\leq 1253$ ) and the patient populations heterogeneous, consisting of late presenters or a mixture of treatment naïve and treatment experienced patients.

Our findings regarding the CD4:CD8 ratio were similar to published small or restricted studies [18-20;23,24;30;31]. Continued increases in the CD4:CD8 ratio for 8 to 15 years since start of treatment have been reported among patients with sustained undetectable viral loads [18;20;23]. Among patients with baseline CD4 counts  $>350$  cells/mm<sup>3</sup>, increases in the CD4:CD8 ratio were partly attributed to decreases in CD8 counts [18-20]. In one study examining CD4:CD8 ratio trends by baseline CD4 count, lower baseline CD4 count predicted higher increases during the first 5 years after start of ART [18]. Higher baseline CD4 count predicted faster time to normalised CD4:CD8 ratio [30] and a greater likelihood of attaining a normalised ratio [18;24;30,31].

In conclusion, there are long-term decreases in CD8 counts and long-term increases in CD4:CD8 ratios, among patients who start ART with CD4 count as low as 50-199 cells/mm<sup>3</sup>.

239 However, starting ART at high CD4 counts is paramount for attainment of a maximal  
 240 CD4:CD8 ratio.

241

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252

## 253 **Conflicts of interest**

254 Dr. Costagliola reports potential conflicts of interest that are outside the submitted work.  
 255 Grants from Janssen-Cilag (2014), Merck-Sharp & Dohme-Chibret (2017), ViiV (2015),  
 256 personal fees from Janssen-Cilag (2016), Merck-Sharp & Dohme-Chibret (2015) for lectures,  
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 258 travel/accommodations/meeting expenses, personal fees from Gilead France from 2011 until  
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261 Remaining authors report no potential conflicts.

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Table 1. Characteristics of the 39,979 patients eligible for analysis

	Pre-treatment CD4 count (cells/mm <sup>3</sup> )						
	<25	25-49	50-99	100-199	200-349	350-499	≥500
Number of participants	2510	1779	3018	7455	14141	6593	4483
Median (IQR) <sup>a</sup> age (years)	38 (12)	40 (13)	39 (14)	39 (14)	38 (14)	38 (14)	37 (15)
Male %	76	75	73	70	72	73	70
Route of HIV infection %							
Homo/bisexual	27	31	33	34	41	45	43
IDU	11	11	11	11	9	9	9
Heterosexual	51	46	45	44	41	37	37
Other/not known	11	12	11	10	9	10	11
Median (IQR) <sup>a</sup> baseline viral load (log <sub>10</sub> copies/ml)	5.30 (0.79)	5.31 (0.81)	5.14 (0.85)	4.91 (0.94)	4.65 (1.00)	4.49 (1.35)	4.01 (2.57)
Median (IQR) <sup>a</sup> baseline CD8 (cells/mm <sup>3</sup> )	340 (320)	480 (421)	610 (501)	749 (540)	900 (594)	981 (674)	1062 (715)
Median (IQR) <sup>a</sup> follow-up (months)	64 (78)	64 (78)	61 (74)	62 (66)	50 (59)	41 (69)	47 (81)

<sup>a</sup> IQR: Inter-quartile range